Early Journal Content on JSTOR, Free to Anyone in the World

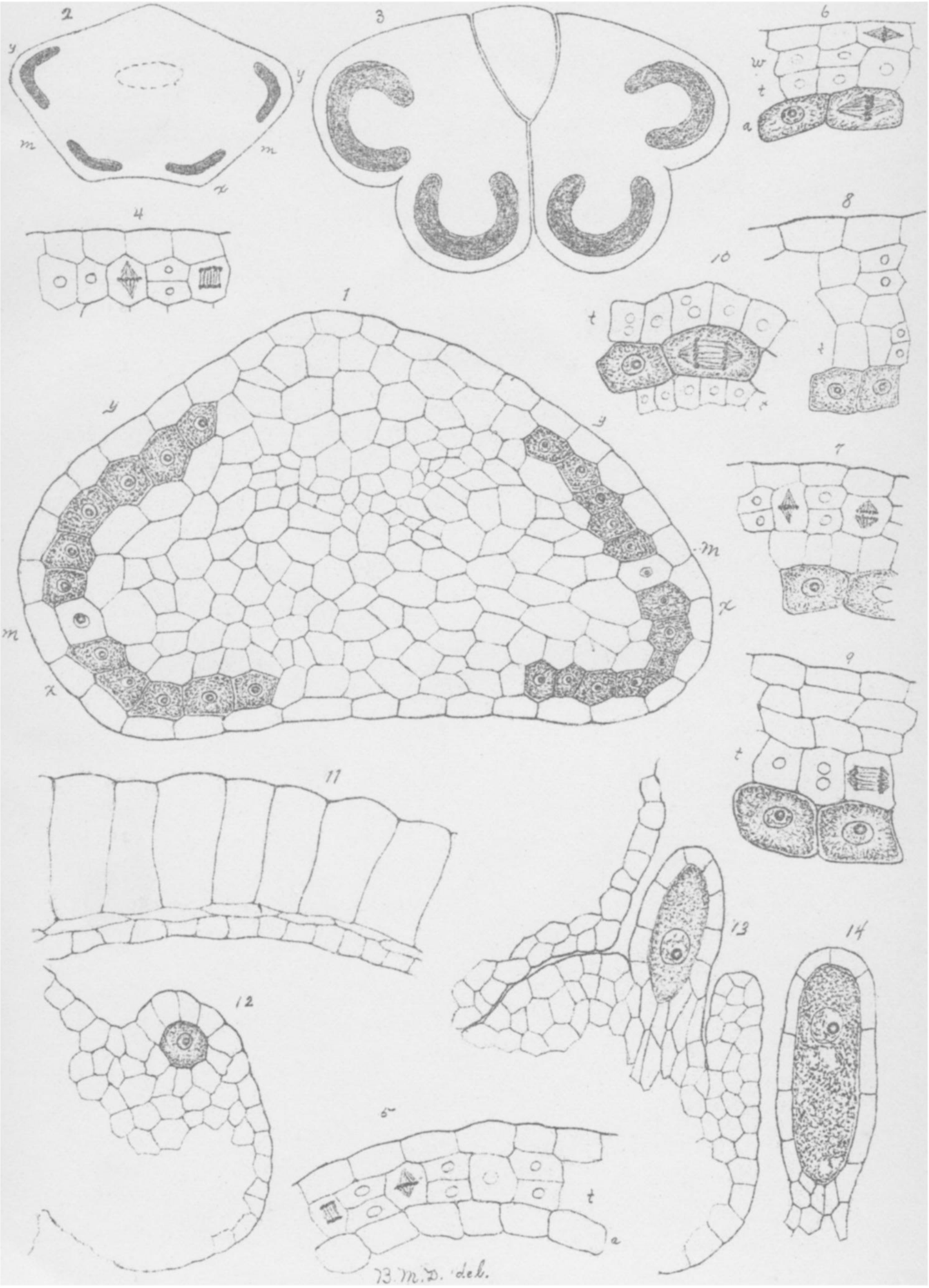
This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

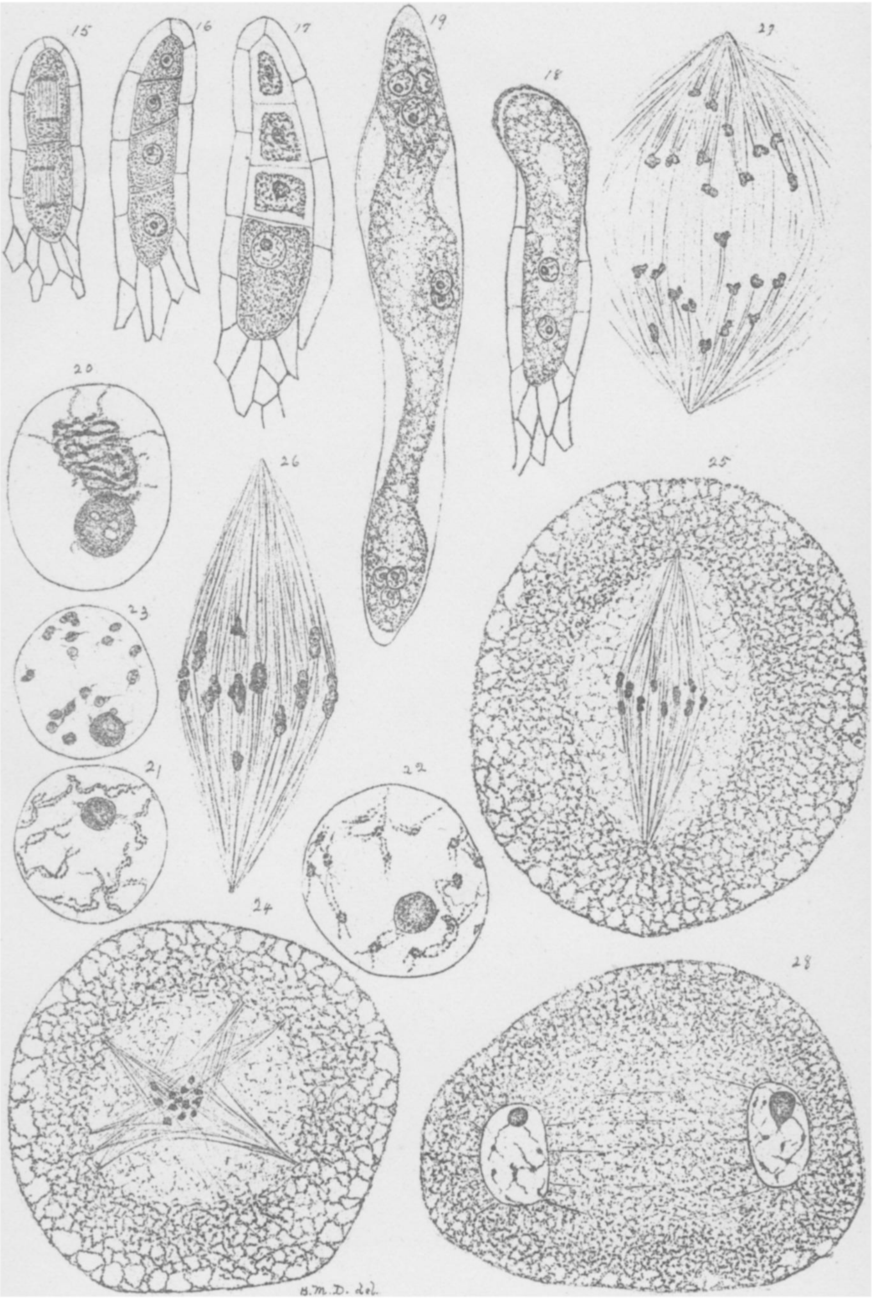
We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

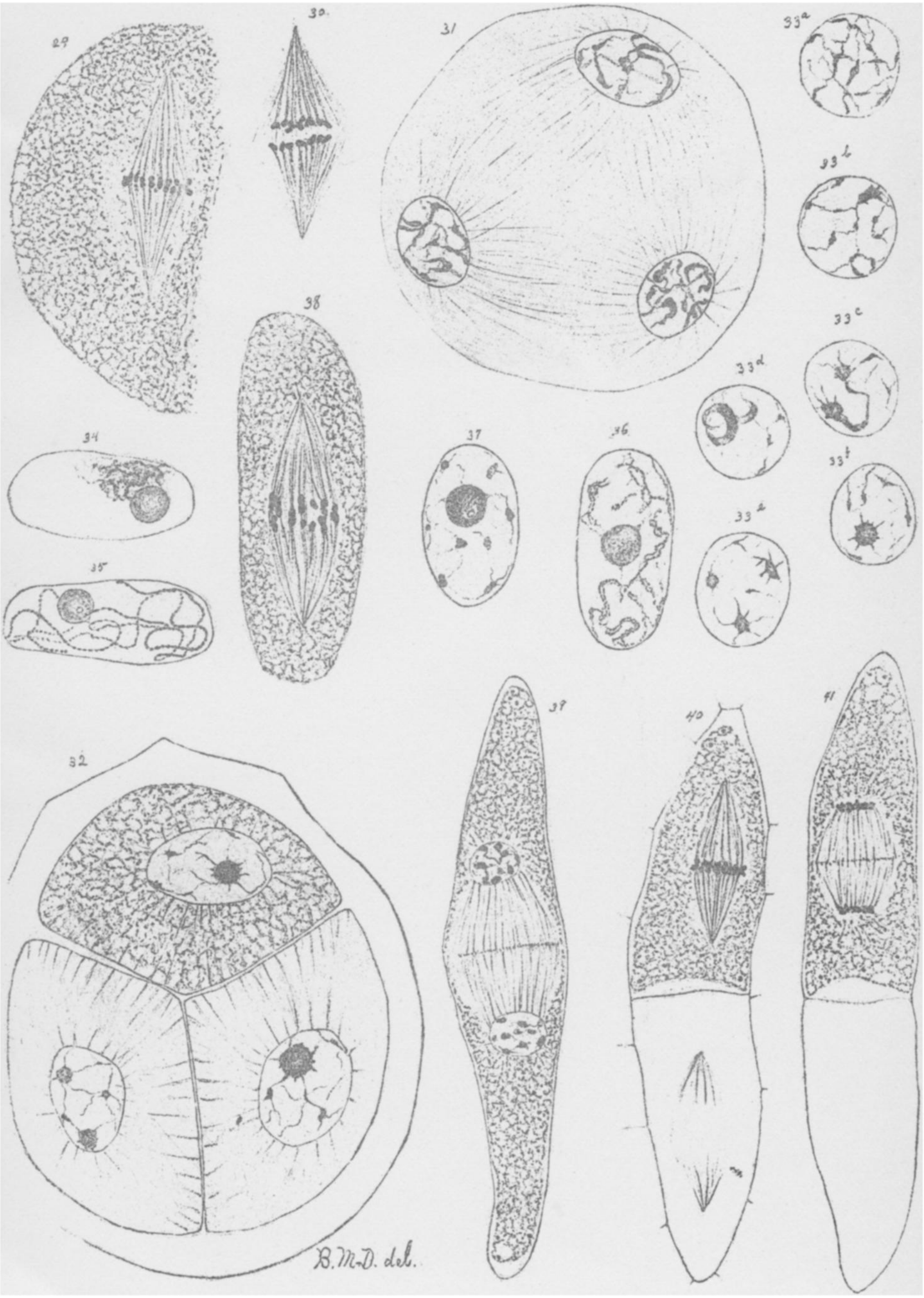


BIGNONIA VENUSTA.



BIGNONIA VENUSTA.

THE HELIOTYPE PRINTING CO., BOSTON



BIGNONIA VENUSTA.

THE HELIOTYPE PRINTING CO., BOSTON

VOL. 26 NO. 3

BULLETIN
OF THE
TORREY BOTANICAL CLUB

MARCH 1899

On the Development of the Pollen Grain and the Embryo-sac in Bignonia venusta

BY B. M. DUGGAR

(PLATES 352-354)

SPORANGIAL AND ARCHESPORIAL DEVELOPMENT

The microsporic Archesporium and Sporangium

Before the flower bud opens, median transverse and longitudinal sections of an anther of *Bignonia venusta* show the pollen mother-cells occupying four boat-shaped layers, as seen in cross section in Fig. 3. Each layer is a single cell in depth, and the general form of the archesporial areas somewhat closely resembles that characteristic of Solanaceae, Labiatae, etc., as described by Warming,* and as figured by him for *Datura Stramonium* and *Mentha aquatica.*

In *Bignonia* I have studied the archesporium and its investments in some detail, beginning with the archesporial and wall layer fundament. In Fig. 1, a cross-section of an anther from a bud of 1 to 2 mm. in diameter, it will be seen that the outer layer of periblem in two considerable regions on each side of a radial line is somewhat richer in protoplasmic contents, and it would seem to be already slightly differentiated as a fundament. General placental growth is now largely confined to the regions *x*, *x*. Placental growth, however, soon becomes more marked at *y*, *y*, and so continues until the general form is that of Fig. 2. Even

---

*Warming, E. Untersuchungen über Pollenbildende Phyllome und Kaulome. Bot. Abhandl. Hanstein, **2**: 1–90. 1873.

[Issued 18 March, 1899]

before the latter stage has resulted, the outer layer of periblem in general has become well differentiated, some of the hypodermal cells in the region *m*, *m* have already lost their previous slight differential character (or lose it entirely with further development), and the four regions of Fig. 2 are evident, but not sharply defined. Thus it would seem difficult here to locate the sporangia relative to the surface of the sporophyll on which borne, unless beginning with such a stage as Fig. 2, where the connective, *m*, is already distinctly different from the remaining portions of the hypodermal layer, and might be taken as separating an upper from a lower surface. Engler's * studies on many forms led to the conclusion that in both extrorse and introrse anthers two sporangia are borne on each surface. My observations on *Bignonia* suggested at an earlier stage a common fundament for each pair of sporangia in radial arrangement, and the improbability of any distinction between upper and lower surfaces in the young condition. Moreover, from what we know of stamens which have been partially changed from staminal to purely floral organs, as in *Canna* and in the pond lily, there is no such location of sporangia, it would seem.

The cells of the hypodermal layer at *x*, *x* and *y*, *y*, Fig. 1, divide by periclinal walls into two layers, as in Fig. 5. The inner of these layers rapidly becomes rich in protoplasm, the nucleus increases in size and the cell wall in thickness, and there results the primitive archesporium of about six or eight cells in each sporangial region, in cross section. In the primitive archesporium the cells undergo no further periclinal divisions, and a single layer is maintained until maturity; but at a later period, when the cells have increased in size and peculiarity, a few radial divisions occur, as in Figs. 6 and 10, usually increasing the extent of the layer to about thirteen cells at the middle part.

In the meantime the cells of the wall layer (secondary hypodermal) divide, forming on the inside a layer of cells soon differentiated as the outer tapetum, and on the outside the first true wall layer, Figs. 5 and 6. The next periclinal division throughout the wall layer, Fig. 7, completes the general development on the outer side. However, around the ends of the boat-shaped archesporial masses, there is some irregularity. The cells equivalent to the general tapetum, as well as those of the outlying wall layer, usually divide several times, Fig. 8, so that the terminal portions of the archesporial layer are sunk deeper into the general tissue.

---

* Engler, A., Beiträge zur Kenntniss der Antherenbildung der Metaspermen. Jahrb. wiss. Bot. **10** : 275–316. 1876. Compare IV., "Uber die sogenannten introrsen und extrorsen Antheren, pp. 299–305."

The layer of cells on the inner surface of the archesporium gradually differentiates itself into an inner tapetum, and here also there are no further tangential divisions.

The two general wall layers persist, and there is no fibrillar development of the outer wall layer. At the time of dehiscence of the anther, these layers are compressed, and the epidermis somewhat modified. According to the early work of Purkinge,* Mirbel† and others the endothecium of these writers (final wall layer) is always partially or wholly fibrous. The more extended study by Chatin ‡ upon developing, mature and dehiscing anthers in a large number of orders demonstrated that this generalization could not be made. Moreover, he found that the presence of fibrous structures is not at all dependent upon the natural position of the order. Chatin distinguished three general classes in which no fibrous layer occurred, as follows :

"1. Que les cellules fibreuses manquent, en général, dans les anthères á déhiscence poricide.

"2. Que les cellules fibreuses sont défaut dans un certain nombre d'anthères á déhiscense longitudinale.

"3. Que dans quelques plantes dont les étames paraissent avoir subi un arrêt de développement, sinon morphologique du moins histologique, l' absence de cellules fibreuses coincide avec la mauvaise conformation du pollen."

Thus he found that among dicotyledons the fibrous tissue is entirely absent in Melastomaceae, Vacciniaceae, and Ericaceae. Again, it is absent in members of such widely separated orders as Tremandraceae (*Tetratheca*), Caesalpinaceae (*Cassia*), Ebenaceae

---

*Purkinge, J. E. De cellulis antherarum fibrosis, Vratislaviae. 1830. [Ref. Chatin ‡.]

†Mirbel, Complément des observations sur le *Marchantia*, suivi de recherches sur la métamorphose des utricles et sur l'origine, les développements et la structure de l'anthère et du pollen dans les végétaux Phanérogames. Mém. de l'Acad. royale des Sci. 13 : ——. 1835.

‡Chatin, A. De l'Anthère, Paris. 1870.

(*Diospyros*), Myrsinaceae (*Badula*), Solanaceae (*Lycopersicum*), Acanthaceae (*Thunbergia*), Asclepiadaceae (*Gonolobus*), and Compositae (*Chaetophora*). It is evident that he noted the absence of such fibrous tissue in *Bignonia*, for he briefly refers to the large cells of the epidermis (see Fig. 11) as doubtless aiding in the dehiscence of the anther, these cells increasing in size very rapidly when the pollen approaches naturity.

During the growth of the archesporium, the tapetal cells may divide radially ; but they soon become considerably modified in appearance, and only nuclear divisions occur. The process of nuclear activity in the tapetum are much as Strasburger* has described. The nuclei divide karyokinetically, Fig. 9, but no cell plate is formed. In time the daughter nuclei may move together, touch, and become flattened against each other, thus appearing as if in the process of direct division. In *Bignonia*, however, the nuclei often remain apart throughout.

It is of some interest to note the rate of growth in the cells of the archesporium, beginning even with the outer periblem layer which gives rise to the archesporium by its first periclinal divisions, measuring the time by the development of the archesporial investments, etc.

The following table indicates the measurements :

TIME.	ARCHESPORIAL.		
	Cell.	Nucleus.	Nucleolus.
Periblem layer first differentiated........................	9.5–13×11–13 μ	5.57 μ	2.8 μ
After the outer tapetum is cut off........................	11–15×16.5–19 μ	8–9.6 μ	4–4.8 μ
Immediately after the final division of the wall layer.	16.5–19×22–27.5 μ	11-14 μ	5.5 μ
During synapsis of the definitive archesporial cells..	30–44×44–60 μ	16.5–18×16.5–22 μ	6.9 μ

After synapsis there is little growth in the pollen mother-cells until divisions begin, although during the spirem stage the nucleolus often attains a general diameter of 8 μ, and a long diameter even greater.

Nucellus and Integument

The ovule development conforms quite closely to that characteristic of most Gamopetalae. The protuberance arising from the placental tissue is a small mass of parenchymatous cells, and in this mass of tissue, growth is much more rapid on one side than on the other, consequently giving the anatropous ovule. In the nucellar mass a large hypodermal cell is soon differentiated as the primitive archesporium. It is readily distinguished by its greater size and by the richness of its protoplasm, see Fig. 12. As this archesporial cell elongates apically, it is enveloped in the epidermal layer alone, and there is no further nucellar development. The funiculus is considerably enlarged, and the cells beneath the archesporial cells are considerably elongated, as in Fig. 13. The single thick integument develops from below these elongated basal cells. During the subsequent expansion of the archesporium into four cells, the nucellar cells are greatly compressed and stretched, as in Fig. 16. Finally, the growth of the embryo-sac causes the complete disorganization of these apical nucellar cells, which disintegrate as the embryo-sac pushes itself out to such an extent as to encroach upon the cells of the integument. Meanwhile, the cells at the base of the embryo-sac become thick-walled and further elongated.

---

* Strasburger, E. Theilungsvorgänge der Zellkerne, 99–100. 1882.

Macrosporic Archesporium

In the microsporangia, both of the divisions of the pollen mother-cells are complete before there is any differentiation in the macrosporangium of the primitive archesporium, or initial cell. When first recognizable as the primitive archesporium, this hypodermal cell is about 15 μ in length. No tapetum is cut off, and growth is rapid. At the time of synapsis in the nucleus of this archesporial cell, the latter is 45 μ in length, and its size, when the spindle begins to form, is about 60 μ. The cell is rich in protoplasm, sometimes with a single vacuole in the vicinity of the nucleus. Owing to the narrow transverse diameter of the cell, the nucleus is often oblong in form. During the formation of the first spindle, the transverse diameter of the cell increases appreciably, Fig. 39. The two equivalent cells resulting from the first division rapidly divide again, usually synchronously in all details, Fig. 15, and there result the four highly differentiated and equivalent cells regarded as potential macrospores, as in Fig. 16. As a rule, the fourth cell of this axial row immediately begins to enlarge at the expense of the others, as shown in Fig. 17. I have noticed several instances in which the third cell retained its normal appearance later than the first two; but ultimately the fourth cell develops the embryo-sac.

In having a single axial row of four cells, and in possessing no tapetum, *Bignonia* agrees with such monocotyledons as *Sisyrinchium iridifolium* and *Hemerocallis fulva* (usually), according to Strasburger*; and among gamopetalous dicotyledons, so far as I have found from literature accessible, with all that have been studied, namely, with Compositae* and Labiatae*; also with some Ranunculaceae† and Berberidaceae.‡

Embryo-sac

The embryo-sac develops by the immediate growth of the fourth or lowest macrospore in the axial row, as mentioned. Since reduction has already taken place, its nuclear divisions are of less interest. The embryo-sac is late in developing, and it is not mature when the flower is fully open. As a rule, it seems to become unhealthy in this conservatory material after the first or second division. The nuclei are small, and divide rapidly. Generally, the embryo-sac became disorganized so readily, and latest ages were so difficult to secure, that lack of effective pollination was suggested as a possible cause of the difficulty. Artificial pollination with pollen from the same plant was ineffective, and attempts were futile to secure in good condition pollen from other plants in distant greenhouses. Embryo-sacs with two and with four nuclei were more commonly seen, as in Fig. 18; but only in a single instance was a mature embryo-sac found, and this was perfectly normal, with the characteristic eight nuclei, as shown in Fig. 19. The three antipodals are free, with no indications of disintegration, the polar nuclei are beginning to fuse, and the sexual nucleus has taken up its position immediately below the two synergids.

---

* Strasburger, E. Angiospermem und Gymnospermen.

† *a.* Mann, L. E. The Embryo-sac of *Myosurus minimus*. Trans. and Proc. of Bot. Soc. Edinburgh, **29**: 351. 1892. *b.* Mottier, D. M. Contributions to the Embrology of the Ranunculaceae. Bot. Gaz. **20**: 241, 296. 1895.

‡ Andrews, F. M. Development of the Embryo-sac of *Jeffersonia diphylla*. Bot. Gaz. **20**: 423. 1895.

DEVELOPMENT OF THE POLLEN GRAIN AND OF THE DEFINITIVE ARCHESPORIUM.

Development of the Pollen Grain

Very early in the development of the microsporangium the definitive archesporium is differentiated, and the nuclei of the pollen mother-cells enter upon a considerable period of rest and growth. As previously mentioned, the few divisions which have occurred in the primitive archesporium are truly of the vegetative or homotypic character, and very little of interest is connected with them. The chromosomes are then small, oval or oblong, and number about fifty, the full number of the sporophyte generation.

The Resting archesporial Cell and its Nucleus

In general, from the time of the last divisions in the primitive archesporium until synapsis of the definitive cells, the pollen mother-cells increase in size to at least twice their former diameter, the nucleus is about two-thirds larger, and the nucleolus approximately one-half greater in diameter. The nucleolus, however, is now larger than the original nucleus of the early differentiated periblem layer.

In the resting cell the cytoplasm is closely netted and the nuclear membrane distinct. The nucleolus takes the gentian stain in the Flemming combination. It is a striking feature in these nuclei that there is very little chromatin on the reticulum, and the nucleolus takes the chromatin stain constantly. In the early period of growth the nucleolus shows a deeper-strained outer zone. This is more evident in the early stages, but before the time of synapsis it is no longer noticeable. Small clear spaces in the nucleolus, which have been termed vacuoles, are present. I have generally been able to trace to these spaces linin strands of the nucleolus, as in some other plants studied later ; and it seems highly probable that the appearance of small vacuolations are often only projections on the surface. This occurs not only during the reticulum stage, but also later.

The reticulum is more closely interlaced in the periphery of the nucleolus, and upon it are found slight thickenings and granules which give up their gentian readily. In addition, there are found a few spherical chromatic masses at definite points on the linin, staining deeply with the gentian.

Under the lower powers the cytoplasm has a granular appearance, and under very high magnification it is very closely netted. In *Bignonia* the cytoplasm is very dense, and as a consequence kinoplasmic radiations or fibers are not so readily distinguished, and probably some effects, especially in later stages of division, are thus obscured.

Synapsis

On passing into the condition of synapsis, Fig. 20, the linin framework is somewhat thicker than in the resting condition. I have observed no double row of chromatic granules nor any indication of fission previous to the contraction of the thread. As usual, during synapsis the contracted linin mass stains poorly, taking the orange diffusely. The coils of the contracted mass are not entirely obscured by the density of the ball, but the diffuse staining renders problematical any full account of changes that may occur during this period.

The nucleolus is at one edge of the contracted thread, and from the optical periphery there are one or more projections on its surface. Several so-called vacuoles may be seen in the middle part, but these also often represent projections, sometimes swollen, leading to linin attachments. Sometimes these structures are refractive. In this stage the nucleolus of the tapetal cells shows some signs of disintegration, and upon its surface there appear very large and refractive clear spaces. These undoubtedly enclose air in some way, and by long treatment with xylol and alcohol, as suggested by Zimmermann,[*] or even with treatment by xylol alone, these vacuoles largely disappear. They disappear at least so far as the air present is concerned, but certain clear spaces remain as before, showing no special refractive power.

The Spirem Stage and Segmentation

No indication of a true spirem is seen until after synapsis, and the initiation of this spirem is marked by a stronger reaction to the stains. At first the spirem consists of a loose, slightly thickened

---

[*] Zimmermann, A. Morphologie und Physiologie des Zellkernes, 41.

thread with many small granules, and a few larger ones. At this stage I have found no indication of distinct rows of granules. They are evidently rapidly formed, however, for somewhat later there is a general splitting of the ribbon with its numerous granules (as in Fig. 21), the parts of which may be adjacent for some distance, and separating widely in other places. The divided thread becomes greatly looped and twisted, apparently making definite bends upon itself in certain regions, but there does not seem to be segmentation in the sense that the entire ribbon falls into distinct segments, the whole extent of each segment entering into a single chromosome (see Fig. 22). Nevertheless, each bent or loop-like portion resulting is undoubtedly the basis of a chromosome, and in a certain view the loop formation is very evident. The figures indicate that we may have a process closely parallel to what is described in the Hepaticae.* The resulting chromosomes often appear spherical, with a few projecting edges. They may seem to be made up of two or three chromatic masses closely fused; and again, or from another view, there is an evident concavity on one surface (Fig. 23).

Throughout this period, also, minute linin attachments connect the chromosomes and the nucleolus, and the nucleolus is often drawn out into a fusiform condition. Some abnormal nuclei occur in which the nucleolus is actually drawn out into the form of a ribbon.

In several anthers I have found two nuclei in many pollen mother-cells during the spirem stage. They are usually abnormal in form, but there has been no indication of how the division has been effected.

The First Division

On the disappearance of the nuclear membrane the kinoplasm of which it is composed opens into the nuclear hollow, the kinoplasmic threads of the membrane being apparently the first to be attached to the chromosomes. Soon, however, the kinoplasm enters from all directions, and it is drawn into the form of a truly multipolar spindle, as often described (see Fig. 24). The general axis of the spindle is finally determined; so that it becomes multipolar in one general plane, and then by a gradual contraction process it becomes bipolar.

---

* Farmer, J. B. On Spore-Formation and Nuclear Division in the Hepaticae. Ann. Bot. 9: 469–523. 1895.

The chromosomes seem to increase in size just before the disappearance of the nuclear membrane. On this entrance of the kinoplasmic fibers there is no indication of the nucleolus, and I have no evidence of the manner of its disappearance.

During the later stages of spindle formation the chromosomes seem to change their form, possibly the splitting having already begun. When they are finally brought into an equatorial region, the alignment is not perfect (Figs. 25, 26), and the chromosomes are much scattered. This is characteristic of all first divisions in the production of the reduced number of chromosomes. The dense cytoplasm is apparently repelled from the spindle region, and only the poles of the completed spindle reach into the denser zone (see Fig. 25), thus somewhat resembling the condition found in *Hemerocallis fulva.** In *Bignonia*, however, the spindle fibers are very numerous, and they terminate in a more distinct apex. The spindle is very large for the total chromatin mass concerned. In this plant the nuclei are much larger than in others under study at the same time, but the chromosomes are much smaller.

Details of reduction phenomena were not followed, since the plants were not suitable for this purpose, but some especially interesting nucleolar phenomena were observed.

After the separation into the daughter segments the chromosomes show a very slight indication of a V-form, by short projections pointing towards the equator, as in Fig. 27. They pass to the poles in scattered and unaligned array, so that at this stage it is relatively an easy matter to estimate the number of chromosomes. From several counts I have concluded that there are twenty five, although twenty four and twenty six have been counted. In the very thin sections necessary for the study of these divisions the knife usually passes through some of the chromosomes, and from this results the only difficulty in counting them accurately.

---

* Juel, H. O. Die Kerntheilung in den Pollenmutterzellen von *Hemerocallis fulva* und die bei denselben auftretenden Unregelmässigkeiten. Jahrb. wiss. Bot. **30**: 205–226. 1897.

After the daughter nuclei are formed the dense cytoplasm rapidly fills the original nuclear hollow, and a very delicate spindle remains. In the dispirem of the daughter nuclei the chromosomes become very irregular in outline, and gradually diminish in size, while there is being gradually formed a large nucleolar-like body with irregular outlines (see Fig. 28). This body takes the chromatic dyes, as did the nucleolus generally before. Afterwards, it seems that this body resulting from the fusion of the chromatic masses is hardly fully differentiated as a nucleolus before the chromatin is again rapidly deposited upon the linin, and the chromosomes are again differentiated for the second division. Here there is evidence of an interesting connection between the chromatin content and the nucleolus.

The Second Division

With the disappearance of the nuclear membrane, the chromosomes prepared for the second division are short and irregularly oblong, broader from one side view than from the other, apparently, and along the middle line of the broader side there is indication of a fission. I have not observed all stages in the formation of the second spindle, but in general it seems to be the same as in the case of the first spindle, except that the second is much more rapidly formed. The second spindle is much narrower than the first, and it is composed of relatively few bundles of fibers, rather than of the loose network of the first division (see Fig. 26). The fibers are more in the form of compact bundles, however, than in the first division (see Fig. 29). In this division there is also a fairly distinct nuclear hollow remaining. Arranged on the nuclear plate, the chromosomes have their long diameter in the plane of the equator, and separation is along the line of fission previously indicated, so that the resulting daughter segments are small and oval in axial view, and somewhat bacilloidal in polar view. Contrary to the condition in the anaphase of the first spindle, these daughter chromosomes move to the poles in a definite line, as in Fig. 30. The remaining central spindle is also composed of a small number of delicate fibers, and the spindle space is rapidly occupied by dense cytoplasm. With the formation of the nuclear membrane, a few polar radiations are evident, and the whole cytoplasm shows the effect of a general radial arrangement, from which there is soon differentiated the complex spindle characteristic of dicotyledons (Fig. 31).

In the daughter nuclei it is again evident that the chromosomes first become irregular and slightly fused, as in Fig. 31, and gradually the fusion continues, or by some process it is returned to one or to several chromatic masses. Where there are several large masses first formed, the fusion of these into a single mass is not always evident; but eventually one prominent nucleolus results, as in Fig. 32, thus agreeing with the condition found in the first division. This gradual mergence of the chromatin mass into characteristic forms has been carefully followed, and various stages in the return of the chromatin from the chromosome state to that of the large nuclear masses are shown in Fig. 33. This mass is at first irregular in outline, but in time this irregularity is lost. One or more linin attachments persist, and everything indicates that the nucleolus of the microspore nucleus has thus resulted from the direct or indirect fusion of chromatic material used in division.

The Microspore

The forming microspores become invested with walls of their own, even the cell plates of previous divisions disintegrating with the general wall of the mother-cell. When first set free, the microspore is somewhat elliptical in form, the nucleus small, and the nucleolus relatively large. It remains in the resting condition for some time, and undergoes a period of growth, during which time the tapetal cells disintegrate rapidly. Until the divisions in the pollen mother-cells are begun the tapetal cells have undergone no disintegration, although becoming granular in appearance and staining deeply. The mature microspore is invested with a very thick wall, and owing to lack of stages and some difficulties with fixing, I have not been able to study the division of the microspore nucleus. As a result of this division, however, the pollen grain contains a larger vegetative nucleus, and a smaller more chromatic generative nucleus. The latter is not separated in a daughter-cell by a permanent cell wall, for in the mature pollen grain it is a free nucleus. Division of the generative nucleus does not take place previous to germination.

DIVISIONS IN THE FORMATIONS OF THE AXIAL ROW OF THE NUCELLUS

Bignonia has not proved a very favorable subject for the study of the axial row divisions in the ovule. Besides being very small, the ovules are not readily separated from the placental attachments, and it is almost necessary to section the entire ovary. Moreover, the developing ovules are bent, and it is difficult properly to orient them so as to have the archesporial axis in a desired plane. Innumerable sections have afforded me only some of the salient stages, sufficient, however, to demonstrate the general method of the reduction divisions. It was very manifest that the reduction divisions did not take place in the embryo-sac, owing to the absence of the characteristic prophase appearance in its nucleus. Moreover, since all of the gamopetalous dicotyledons yet worked upon have no tapetum and only an axial row of four cells developed from the archesporial cell,* in any of these plants where such uniformity seems to exist it seemed of interest to study the divisions in the formation of the axial row. Details of the general macrosporic development have already been given, and here only division phenomena will be briefly discussed.

The resting nucleus of the initial cell is at first much like that of the underlying growing cells of the nucellus. The nucleolus is then relatively small and readily loses the gentian stain for the orange. There is a loose reticulum of linin threads, and upon this a considerable number of large granules taking the chromatin stain.

As in the nuclei of the pollen mother-cells, synapsis is manifest at an early prophase stage. It is marked by the usual contraction of the linin ribbon, which is always in contact with the nucleolus on one side. The whole mass is usually in the center of the oval nucleus, the nucleolus in this case being in contact with the nuclear membrane, as in Fig. 34.

The archesporial cell has attained considerable length when the nucleus enters upon the spirem stage. The return from the condition of synapsis is especially marked by much better staining in the general ribbon. The ribbon is somewhat thicker than before, and the few large chromatic granules are replaced by a more chromatic ribbon, and by many more very evident granules, giving to the ribbon a nodulate appearance, as in Fig. 35. Moreover, the ribbon is coiled and looped in definite curves; and it may be readily seen that there are no anastomoses of the general thread.

---

* Strasburger, Angiospermen und Gymnospermen.

The process of chromosome formation seems to follow the general method outlined for the first division of the pollen mother-cell, except that here no such definite loops or rings have been observed. Radiating from the nuclear membrane in the direction of the axis, especially, are kinoplasmic threads. The disappearance of the nuclear membrane initiates the formation of a loose spindle in every way equivalent to the one first formed in the pollen mother-cell. The chromosomes are again irregularly scattered in the region of the nuclear plate, and the characteristic heterotypic division of this plant is unmistakable (Fig. 38). In this case, however, there is no large space free from trophoplasm immediately surrounding the spindle. I have also been able to estimate the number of chromosomes in this division, and it corresponds to the reduced number of the male archesporium, about twenty five.

After the formation of the daughter nuclei, the chromosomes may be identified for a time (Fig. 39), but as no later telophase stages were found, I have no notes concerning the reappearance of the nucleolus, or the early stages in the formation of the second spindle. When the second spindle is complete (Fig. 40), a glistening cell wall separates the two daughter-cells, there is no indication of the former spindle fibers, and the cytoplasm is contracted or repelled from the newly-formed wall. The chromosomes are arranged at the nuclear plate in a definite plane, and the characteristic homotypic division is evident. They separate longitudinally, and the bacilloidal daughter segments pass to the poles on a definite alignment, as in the corresponding division in the microsporic development.

There is every reason to believe that these divisions are truly homologous with the two divisions in the pollen mother-cell, and that here we have the reducing divisions preceding the formation of the female sexual nucleus. Of the monocotyledons yet published upon, species of *Allium*[*] show reduction, or the indication of reduction, in the division of the initial cells ; and among dicotyledons *Helleborus foetidus* and *Podophyllum peltatum*[†] show the reduced number of chromosomes during the formation of the axial row.

Notes on Material and on Methods

Material of *Bignonia venusta* in quantity was secured from the botanical conservatories. A single cluster of flower buds will give many stages of development; but when it is desired to have material showing division in the pollen mother-cells, it is quite necessary to examine an anther from each bud in order to avoid loss of time in sectioning useless material. At first I experienced some difficulty in securing such stages from collections made during bright forenoons. On examining a number of buds it was easy roughly to locate the beginning of division by the bursting of the calyx lobes. By this means, during the early afternoon I located and marked about twenty buds apparently just preceding division. Half a dozen buds of the same stage were then examined as checks, and found to be just preceding the formation of the spindle. At ten o'clock the following morning the marked buds were examined and all of them had passed through both divisions of the pollen mother-cells. This one experiment, together with previous failures, led me to suspect that these divisions were very rapid in this plant, and probably occurred at night. Material collected at night did yield many divisions, but at noon on a cloudy day I likewise secured all material desired of these stages. Nevertheless, it may be of interest that these divisions were never found during bright days.

Fixing, embedding, etc. In general, the material studied was fixed in Flemming's solution, as this proves so generally satisfactory in work with plant tissues. The mixture is substantially Flemming's strong solution, and the formulae are given in percentages, and as made up for ½ gram osmic acid :

---

[*] Strasburger, Ueber Kern und Zellteilung im Pflanzenreich, Histol. Beitr. H. I., 243.

[†] Mottier, D. M., Ueber das Verhalten der Kerne bei der Entwickelung des Embryosacks und die Vorgänge bei der Befruchtung. Jahrb. wiss. Bot. 31: 125–158. 1897.

16 c.c.	1%	Chromic acid	or	1⅓ grams
3 c.c.	2%	Osmic acid		½ grams
1 c.c.		Glacial acetic acid		8⅓ c.c.
		Water, about		160 c.c.

The Flemming triple stain of safranin, gentian violet, and orange was used to some extent. After much experimentation, however, it was found advisable to leave out the safranin. Gentian of the full strength recommended gave good results in chromosome differentiation ; but the spindle structures were not then well stained. For spindle structures, and for the differentiation of the kinoplasm, best results were obtained by the use of a very weak gentian, in which the sections were stained from twelve to twenty-four hours.

Explanation of Plates.

PLATE 352.

All figures were drawn with the aid of an Abbè camera lucida, projection 30 cm., tube length 15.5 cm., Leitz oculars 3 and 8, and objectives 7 and $\frac{1}{16}$ (hom. imm.) were used.

FIG. 1. Cross section of young anther showing early differentiation of periblem layer, from which the archesporium and wall layers are eventually derived.

FIG. 2. The four sporangial regions well differentiated ; later than Fig. 1.

FIG. 3. Outline of mature anthers in cross section.

FIG. 4. Division of the hypodermal layer forming primitive archesporium within.

FIGS. 5 and 6. Formation of the first true wall layer without, and the outer tapetum within.

FIG. 7. Development of the two wall layers.

FIG. 8. Extra growth around the ends of the archesporial regions.

FIG. 9. Nuclear division in the tapetal cells.

FIG. 10. Increase in extent of the archesporial layer by anticlinal divisions of the vegetative type.

FIG. 11. Epidermis and wall layers at the maturity of the anthers.

FIG. 12. Differentiation of the hypodermal cell as the initial cell of the macrosporic archesporium.

FIG. 13. Growth of the archesporium and development of the integument.

FIG. 14. Archesporial cell immediately preceding the first division.

PLATE 353.

FIG. 15. Second division in the axial row.

FIG. 16. The four cells of the axial row.

FIG. 17. Development of the fourth cell in the axial row at the expense of the others.

FIG. 18. Developing embryo-sac with two small nuclei.

FIG. 19. Mature embryo-sac with synergids and egg-cell, fusing polar nuclei, and antipodals.

FIG. 20. Nucleus of the pollen mother-cell in synapsis.

FIG. 21. Longitudinal division of the spirem thread.

FIG. 22. An early stage in the differentiation of the chromosomes in the nucleus of the pollen mother-cell.

FIG. 23. Nucleus with mature chromosomes.

FIG. 24. Formation of the multipolar spindle.

FIG. 25. The complete spindle of the pollen mother-cell showing scattered chromosomes and nuclear hollow.

FIG. 26. An enlarged view of a spindle in the same stage as in the preceding figure.

FIG. 27. A characteristic spindle in the anaphase of division.

FIG. 28. A telophase of the first division in which the chromatic substance is largely fused into a nucleolar-like body.

PLATE 354.

FIG. 29. Compact second spindle of the pollen mother-cell.

FIG. 30. An anaphase stage of the second division.

FIG. 31. A telephase of division before the formation of a cell plate.

FIG. 32. An early stage in the differentiation of the microspores.

FIG. 33. Various appearances of the chromatin content in the dispirem of the second division, preceding the formation of a nucleolus.

FIG. 34. Synapsis in the nucleus of the axial-row mother-cell.

FIG. 35. A spirem stage in the nucleus of the axial-row mother-cell.

FIG. 36. A nucleus showing longitudinal division of the spirem thread.

FIG. 37. A nucleus with chromosomes well formed.

FIG. 38. The first division in the macrosporic archesporium.

FIG. 39. A telephase stage in the same division.

FIG. 40. Complete spindles of the second division in the macrosporic archesporium.

CORNELL UNIVERSITY, ITHACA, N. Y.